Master thesis **2021:06**
Department of Forest Ecology and Management

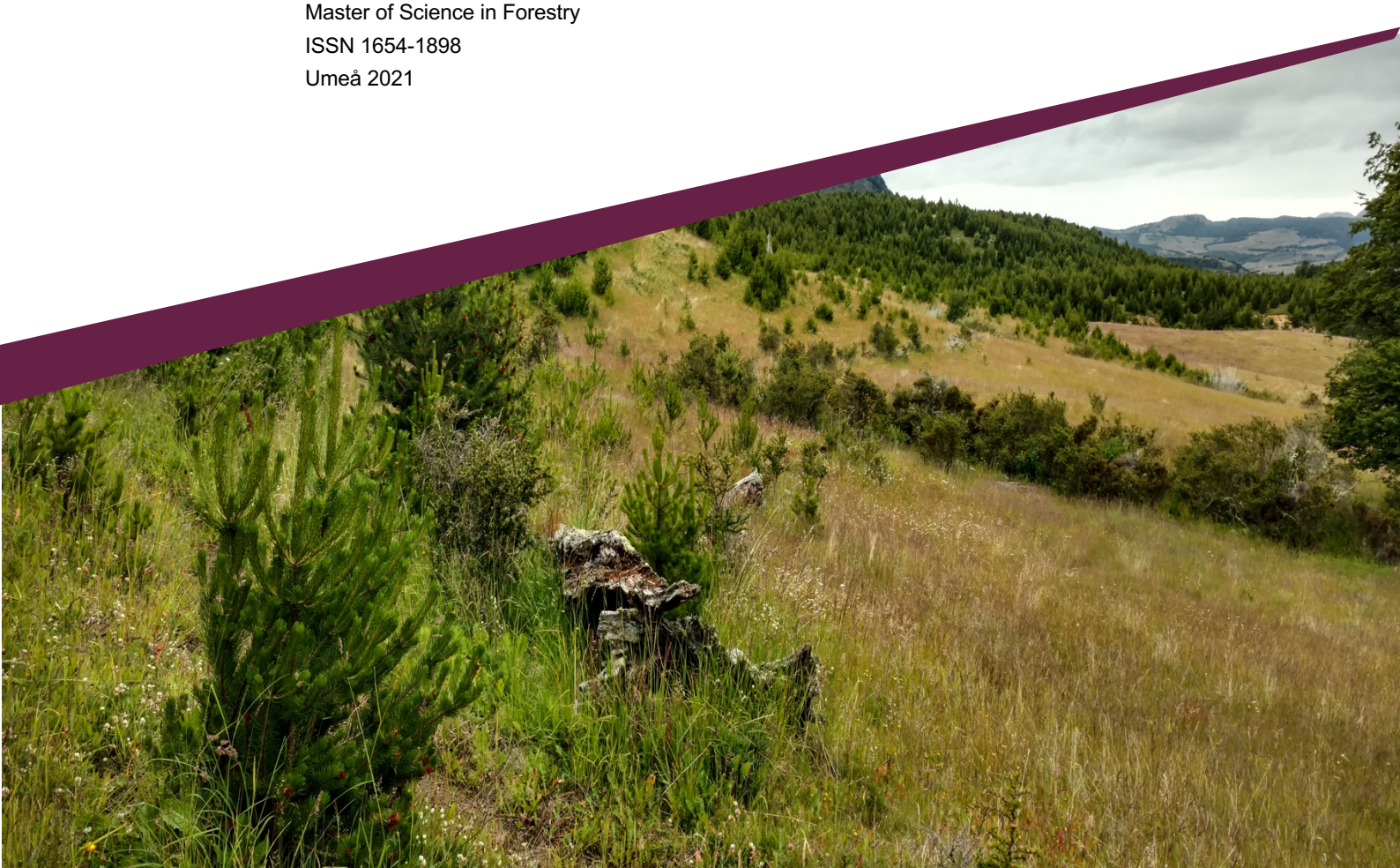
Trait variation of Lodgepole Pine

– do populations differ in traits depending on if they are invasive or in their home range?

Variation av egenskaper hos Contortatallen- har invasiva och inhemska populationer olika egenskaper?

Lisa Lindberg

Master thesis • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Forest Sciences
Master of Science in Forestry
ISSN 1654-1898
Umeå 2021



Trait variation in Lodgepole Pine – do populations differ in traits depending on if they are invasive or in their home range?

Variation av egenskaper hos Contortatallen- har invasiva och inhemska populationer olika egenskaper?

Lisa Lindberg

Supervisor:	Michael Gundale, Swedish University of Agriculture, Department of Forest Ecology and Management
Deputy supervisor:	Susan Nuske, Swedish University of Agriculture, Department of Forest Ecology and Management
Deputy supervisor:	Paul Kardol, Swedish University of Agriculture, Department of Forest Ecology and Management
Deputy supervisor:	Marie-Charlotte Nilsson Hegerthorn, Swedish University of Agriculture, Department of Forest Ecology and Management
Examinator:	Sandra Jämtgård, Swedish University of Agriculture, Department of Forest Ecology and Management

Credits:	30 credits
Level:	A2E
Course title:	Master's thesis in forest science (or biology, or soil science) at the Department of Forest Ecology and Management
Course code:	EX0958
Program:	Master in Forest Sciences
Department:	Department of Forest Ecology and Management
Place of publication:	Umeå
Year of publication:	2021
Cover image:	Susan Nuske
Title of series:	Master thesis
Part number:	2021:06
ISSN:	1654-1898
Keywords:	Invasive species, traits, introductions, native, Pinus contorta, Lodgepole pine

Swedish University of Agricultural Sciences
Faculty of Forest Sciences
Department of Forest Ecology and Management

Abstract

As a result of the global world we today live in, species are transported outside of their native range to new areas. Some of these species becomes invasive, which can cause a variety of negative effects. Traits are often thought to play an important role in invasion success. The purpose with this thesis was therefore to test if and how traits of the invasion species *Pinus contorta* varied depending on the subspecies, and where it is growing (i.e. native or introduced range).
Samples had prior to this thesis been gathered from 3 subspecies of *P. contorta* in its native range (USA) and from an unspecified subspecies in plantations and invasion fronts in an introduced range (Patagonia). Traits of sampled needles, seeds and cones were then used as a basis for multivariate and univariate analyses to detect significant differences between the sampled categories. Significant trait differences were found between the three native subspecies: *latifolia, murrayana* and *contorta*. The Patagonian *P. contorta* was found to be the most similar to the native subspecies *murrayana.* Also, the Patagonian *P. contorta* had traits that differed significantly from all of the native subspecies. Overall I found no statistical evidence of a lower trait variation in the introduced *P. contorta* compared to the native. I further found some trait differences between the Patagonian *P. contorta* trees in the plantations and at the invasion front. As a whole my results indicate that invasive species can differ in traits when comparing the species in the native and introduced range, thus providing an example of an invasive species displaying a change in traits compared to their native range. The results can also be used to support several of the existing hypotheses regarding invasion success.

Keywords: Invasive species, traits, introductions, native, Pinus contorta, Lodgepole pine

Table of contents

List of tables

List of figures

Abbreviations

CAP	Canonical analysis of principal coordinates
contorta	*Pinus contorta contorta*
CV	Coefficient of variation
EICA	Evolution of increased competitive ability hypothesis
latifolia	*Pinus contorta latifolia*
murrayana	*Pinus contorta murrayana*
PI	Patagonian *Pinus contorta* at invasion fronts
PP	Patagonian *Pinus contorta* in plantations
SLA	Specific leaf area

1. Introduction

1.1. Background

Invasive species are a common problem across the globe today. As species spread, often by humans, they sometimes establish and become invasive (Williamsson, 1996: Simberloff, 2013). Global trade and shipping have resulted in an increase in the pace of introductions of non-native species to new areas where they become invasive (Meyerson & Mooney, 2007). As invasive species enter new ecosystems, they can cause a variety of negative changes. For example, invasive species can change habitats, alter carbon and nutrient cycles, and compete with native species, sometimes reducing native biodiversity (Sullivan et al. 2007; Ehrenfeld, 2003; Fritts & Rodda, 1998). Apart from the biological effects there are often also other effects which can be more or less apparent as well as hard to measure and define, for example monetary costs and losses (Marbuah et al. 2014). Invasive species ecology is a very active area of research with many research dimensions (Gurevitch et al. 2011). Substantial research is focused on why and how some introduced species can become successful outside of their natural range. Some explanatory ideas are based on external factors affecting species. For example, the enemy release hypothesis suggests species escape natural enemies from their native environment when introduced to new environments, allowing them to perform better (Keane & Crawley, 2002; Mitchell & Power, 2003). In a study by Mitchell & Power (2003), invasive plants were found to have fewer infections from pathogens compared to their native environment, and plants were more invasive when they experienced a higher degree of enemy release.

Another area of invasive species research is more connected with traits. A ´trait´ in the simplest way can be described as a measurable characteristic of an individual or a part of an individual (Violle et al. 2007). Apart from the individual level, some scientists choose to also recognize traits as properties of higher levels, such as traits of populations, species and communities (Drenovsky et al. 2012; Shipley, 2010). Including more levels can be useful when examining invasion ecology since invasions and their effects often are concerning higher levels than only on the individual level. Therefore for this work, all the above-mentioned levels are accepted in the definition of ´traits´. Many ecological theories consider traits as important parts of the theories, especially those traits that often are described as functional traits, since they impact the individuals performance and

fitness (Díaz et al. 2016: Reich, 2014; Violle et al. 2007). Plants are affected by biotic and abiotic conditions and species have evolved different strategies to deal with these conditions. Consequently, trait variation could be the result of differences in plant strategies because of trade-offs and differences in allocation of resources. In a study by Lake & Leishman (2004), invasive plant species were for example found to overall have a higher specific leaf area (SLA) compared to the species found in the communities they were invading, which is a trait indicative of high growth rates. Invasive species thus seem to generally allocate more resources to growth, which seems to be connected to invasion success.

There are many mechanisms that influence an exotic and invasive plant population's traits in their new environments. Traits are properties of a phenotype, which are influenced by the environment (E), genetics (G), and the interplay between the two (GxE) (Geber & Griffen, 2003: Barker et al. 2018). At the most basic level, one genotype can give a variety of phenotypes as a result of environmental influences (i.e., phenotypic plasticity) (Bradshaw, 1965). Traits can therefore also be influenced by the environment (E) a plant is grown in (Geber & Griffen, 2003: Barker et al. 2018), which could potentially cause an invasive or exotic population to have different traits than its native population since they grow in different environments. In addition to the environmental influence on traits, genetics (G) can also determine how traits develop for exotic and invasive species in their new environments (Prentis et al. 2008: Dlugosch & Parker, 2008). Genetic diversity is for example known to affect invasion success. Genetic bottlenecks that often occur when an exotic species initially arrives in a new environment (also referred to as "founder effects") usually decreases the genetic variation within the population. Such events can potentially influence the initial trait values in a new environment, as well as subsequent evolutionary trajectories of traits. The degree to which genetic bottlenecks occur depends on the size of the introduced population (Nei et al. 1975). Invasive species often stem from small populations that were the initial colonizers, thus invasive populations generally should be expected to have lower genetic diversity than populations within the native range (Dlugosch & Parker, 2008). Since genetics influence phenotype and thus trait values (Geber & Griffen, 2003: Barker et al. 2018), one could easily expect that decreased genetic diversity from "founder effects" to also result in decreases in trait variation, though Dlugosch & Parker (2008) found few few studies that support this idea.

In addition to the above-mentioned possible genetical impacts on traits and invasion success, interplays between environment and genetics (G x E) also offers some potential explanations (Prentis et al. 2008: Whitney & Gabler, 2008: Blossey and Nötzhold, 1995: Richards et al. 2006). An example of such an interplay is the rapid selection of certain traits (Prentis et al. 2008). Specific traits which are often explained as connected to invasion success, have been found to sometimes undergo rapid changes after arriving in a new environment; a sort of fast evolution (Whitney & Gabler, 2008). The idea is that by quickly adapting and evolving to its new environment the introduced species is given the potential to more easily become invasive. Furthermore, connected to the rapid selection of specific traits and the enemy release hypothesis is the evolution of increased

competitive ability hypothesis (EICA), whereby it is predicted that exotic species quickly evolve to be more competitive in the absence of enemies, which correspond with a shift in key traits related to growth, reproduction, or defense (Blossey and Nötzhold, 1995). More specifically, it is hypothesized that as an introduced species is released from enemy pressure, it can result in a decrease of defense traits while traits associated with growth and reproduction can develop in an advantageous direction for the species.

Another way that G x E interactions can emerge in introduced populations is when different genotypes differ in their plastic response to their new environment (Richards et al. 2006). Some species or genotypes appear to be better able at quickly responding to a new environment by changing their phenotypical traits. Such plasticity should not be confused with the fast evolution suggested by the EICA hypothesis. Phenotypic plasticity could instead be described as the ability of traits of genotypes to change rather than a process over multiple generations. Some invasion success may therefore be explained by the possibility for a genotype to react to a variety of environments by altering its traits. Consequently, well adapted individuals could have a higher chance of survival and successful reproduction, thus increasing the invasion potential of the species in a new environment. Davidson et al. (2001) found that species associated with invasions often display high phenotypic plasticity, which suggests that phenotypic plasticity potentially plays an important role in invasions. There are clearly numerous hypotheses that can explain invasion success, and many of them may act simultaneously on different scales in time and space (Gurevitch et al. 2011). Many of these hypotheses suggest that key traits may change upon introduction to a new range, which may ultimately influence invasion success or failure in those new environments. By clarifying and increasing the knowledge about what enables some plant species to successfully invade new areas, more efficient measures can be taken for management and avoidance of new invasions.

Pines, which are in the genus *Pinus*, provide many examples of successful invasions globally. Members of this genus originate from the Northern Hemisphere, and have been introduced to many parts of the world for production forestry purposes (Rejmánek & Richardson, 1996: Langdon et al. 2010: Richardson et al. 2008). As pines have been introduced to the Southern Hemisphere many of the species have been classified as invasive in the countries they have been introduced to. Lodgepole pine (*Pinus contorta*) is an example of a pine species which is considered as invasive in several places around the world (Ledgard, 2001: Langdon et al. 2010). Generally, Lodgepole pine is said to consist of four subspecies: *contorta, bolanderi, murrayana* and *latifolia*, which all originated in western North America (Lotan & Critchfield, 1990). Traits have been found to differ somewhat between the subspecies, for example regarding needle and seed size and the level of serotinous adaptation in the cones. However, the subspecies are overall inter-fertile and ecologically and morphologically similar in many aspects. The subspecies of Lodgepole pine have been introduced to many areas around the world, mainly to be used in forestry (Ledgard, 2001: Langdon et al. 2010). Traits said to be general for the species, such as light seeds, short time periods as juveniles, and short intervals between the production of high

amounts of seeds, have been used as possible explanations to why the species is often considered as invasive, as it often spreads from plantations and colonize adjacent areas (Rejmánek & Richardson, 1996: Gundale et al. 2014: Ledgard, 2001). The adjacent ecosystems are often significantly affected and altered (Gundale et al, 2014: Ledgard, 2001). The effects can often lead to ecological and economical costs and losses to many different stakeholders, for example a reduced biodiversity as well as the use of control and eradication measures, which are often associated with invasive species. Therefore more knowledge about invasive Lodgepole pine could be useful for better mitigation and control measures.

1.2. Aim and hypothesis

The intention with this thesis is to examine traits of Lodgepole pine which in many areas of the world is considered as invasive. By evaluating variation in traits within and between *Pinus contorta's* native range (the Pacific Northwest, USA) and invasive range in Patagonia (Chile and Argentina), I aim to bring forward new knowledge about the species and its invasiveness. Within the native range, I aim to determine whether or not there is a difference in traits between three subspecies, and to determine which native subspecies the introduced and invasive Lodgepole pine in Patagonia is the most similar to. Further, I aim to assess whether the range of traits in the introduced range (i.e. Patagonia) differs compared to the native range. I lastly aim towards exploring if there are trait differences between plantation trees and invasion front trees (i.e., trees located furthest away from the plantations) of the introduced *P. contorta* in Patagonia.

I tested the following hypotheses: i) I expected that each of the three native subspecies would exhibit significant trait differences from one another. ii) I anticipated that traits of the introduced and invasive subspecies in Patagonia would be most similar to subspecies *murrayana*. My rationale for this expectation was that there is some anecdotal evidence that subspecies *murrayana* has been introduced to Patagonia[1], likely because of the climate similarities between *murrayana's* native range, which may also cause trait values to be more similar. iii) I expected mean trait values of the introduced and invasive subspecies in Patagonia to deviate from the native subspecies. The basis for this predication is the possible "founder effects" and potential influence of different local environmental conditions (i.e., phenotypic plasticity). IV) I expected the trait variability (i.e., coefficients of variation) to be lower for introduced and invasive populations compared to native subspecies. I expected this because introduction may have resulted in genetic bottlenecks that reduced genetic diversity. V) I anticipated a difference in traits between the populations of Patagonian plantations and Patagonian invasion front due to possible effects of i.e. the EICA hypothesis or environmental differences.

[1] Martín Andres Nuñez, Professor, National University of Comahue (Argentina), personal communication.

By testing these hypotheses, I aimed to increase the understanding regarding invasive species traits and how invasive species can become successful in new environments.

2. Material and methods

2.1. Sites and field sampling

Data analyses for this thesis was based on a set of already collected trait data of Lodgepole pine (*Pinus contorta L.)*. Samples had been collected during 2018 at a total of 32 sites. Within the native range (USA), 24 sites in total were located, including 8 sites for each of the 3 subspecies, *contorta*, *murrayana*, and *latifolia* (Figure 1).

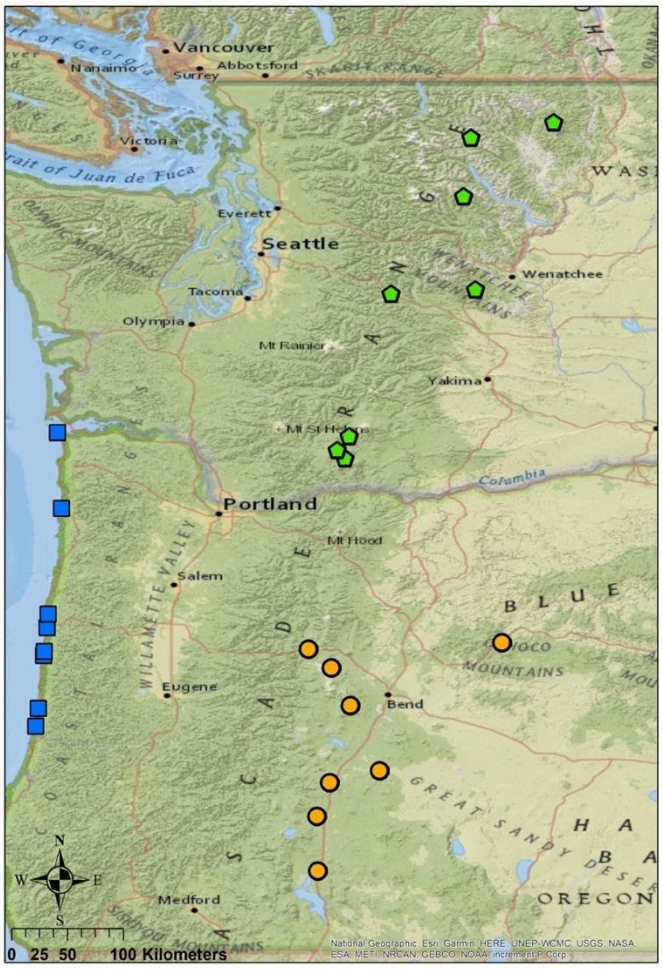


Figure 1.Map of sampled sites of native Pinus contorta in the Pacific North West (USA). Sample sites for the subspecies are displayed as: blue for subspecies contorta, yellow for murrayana, green for latifolia (National geographic et al. 2020).

In the Patagonia region, where *P. contorta* has been introduced, 8 sites were sampled in total, including 3 sites in Argentina and 5 sites in Chile (Figure 2). At these sites, samples were randomly collected from 8 trees within plantations, as well as 8 trees from ´invasion fronts´ stemming from these plantations, for measurement of traits (described below). The "invasion front" consisted of Lodgepole pine trees furthest away from the plantations at each site.

Figure 2. Locations of the sampled sites of introduced Pinus contorta in plantations (National geographic et al. 2020).

Ground accessible (ca. 4 m height) branches containing both brown (>1 year) and green (immature) cones were sampled from each tree on the sun exposed side of the tree (i.e. often the north and south side of the tree in the Southern and Northern Hemisphere, respectively). From each branch, we removed brown and green cones, and fully expanded green needles were collected from the needle whorl from the previous year. For cones, brown cones were collected from the third year whorl, representing cones fully matured during the previous autumn. Green cones were collected from the second year whorl, meaning they were only a few months from opening. After collection, all samples were then dried in an oven at 70 °C for 48 hours, after which leaf trait variables were measured.

2.2. Laboratory analyses and calculations prior to thesis work

Some traits (19 in total) of the collected cones and needles were measured prior to this thesis work. For each trait variable, multiple measurements were made for each individual tree at each site, which allowed the average trait values per site to be calculated. Trait variables were based on needles, seeds and cones. For needle traits, 20 needle fascicles (consisting of 2 needles) from each tree were weighed together and then divided by 20 to get average needle mass per fascicle per tree. Then, 20 needle fascicles were scanned with a flatbed scanner (EPSON Perfection V800/V850 1.9 V3.93 3.9.3.2), and the resulting image was processed with the program WhinRizo 2016 to gain average needle length, projected needle area and surface needle area. Projected area is an area measurement based on the individual needles length multiplied with the width. WhinRizo calculated the total projected area for all measured needles of a tree and this sum was then used to calculate the average needle projected area for each tree. WhinRizo calculated needle surface area by first calculating the area of two sides by multiplying the length with height and 2, to get both sides. Then the total area of the two bordering sides were calculated by multiplying the width with height and then by 2, to get both sides. The end areas were found as the length was multiplied with the width and 2. All these areas were then added up to get the surface area of a needle. Surface areas of all the measured needles for a tree were then summarized and then used to calculate average surface area per tree with the unit mm^2. Samples were ground in a Wiley Mill using a 1 mm particle size mesh. A portion of this ground sample was removed for lignin and cellulose analysis, and the remaining sample was ground to a powder using a ball mill, for analysis of nitrogen, carbon, and phosphorous content. Ground subsamples of needles were sent to Colorado State University (USA) for measurements of percentage of nitrogen, carbon, cellulose and lignin, as well as total phosphorus content (mg kg^{-1}).

For cone measurements, up to 20 cones (when available) were individually measured per tree. Cone measurements included: length, width, and weight. Length, width and weight were measured for mature cones and cones overall. Immature cones were measured for weight. Length and width were measured with a Vernier Caliper. At some sites, not all trees contained 20 cones.

For seed measurements, 20 seeds per tree (if available) were randomly collected from the sampled cones from each tree. The total number of seeds were then weighed together and then the weight was divided by the number of seeds, resulting in an average seed weight per tree. Average seed length, projected area and surface area were calculated by using the same method as for needles with WhinRizo.

2.3. Laboratory analyses and calculations during thesis work

2.3.1. Laboratory work and calculations for Phenolics

During this thesis work I have measured total phenols from the ground needles of the sampled trees (320 samples in total). I used a method for total phenols based on a modified version of the method used by Stern et al. (1996), referred to as the Prussian Blue Technique. I analyzed the samples in a total of 6 batches. From the sampled needles that had been ground prior to this thesis work, I weighed 50 mg from each tree and put the material into test tubes. An extract was then created where I added 20 ml of 50% methanol to the 50 mg of grounded needles in each test tube. The extract was then shaken for 1 hour and then frozen until the next step in the process. Frozen extracts were brought to room temperature, and then centrifuged at 3000 rpm for 5 minutes. Further I diluted the extracts again with 50% methanol to a concentration of 0.04 mg/ml. Again the extracts were then stored in a freezer until used in the analyses. I performed the analyses in 6 batches, with a randomized set of samples from each country in each batch. Extracts for each batch were then brought to room temperature before used in the analyses. A 20 ppm solution was created, where I added 50% methanol to 20 mg of Catechin which is a type of phenol, until the volume was 1000 ml. The solution was then stirred with a magnet on low temperature on a heated magnetic stirrer. From this solution, I prepared 6 standards each time a new batch was about to be analyzed. I placed 0, 0.25, 0.5, 1, 2 and 4 ml of the solution into glass tubes where I further diluted them with 50% Methanol to concentrations of 0, 1, 2, 4, 8, and 16 ppm Catechin, with a total volume of 5 ml for each. Before each batch were analyzed I also created two new reagents. Reagent 1 was created by adding some Milli-Q water to 0.5264 grams of $K_3Fe(CN)$ in a beaker and then stirred with a magnet until the solids were dissolved. I then poured the solution into a volumetric flask and more Milli-Q water was added until the volume of 200 ml was reached. I created reagent 2 by adding some Milli-Q water to a beaker containing 9.64 grams of $FeNh_4(SO)_4$ and then stirring it with a magnet until no solids were visible. The solution was then poured into a volumetric flask where I added 1.7 ml of HCl. I then brought the solution to a volume of 200 ml with Milli-Q water.

For each batch, I put 5 ml of the sample extractions into glass tubes. In intervals of 30 seconds for each standard or sample extracts, I added 3 ml of reagent 1, followed by 3 ml of reagent 2, which was then transferred to a cuvette, and measured on a spectrophotometer at 720 nm. At 30 seconds after reagent 2 was added I noted the absorbance of the sample or standard. I first executed the procedure for the 6 standards and then the prepared sample extractions. The 0 ppm standard was first used to zero the spectrophotometer, allowing comparisons of absorbance values against a base value. I repeated the procedure in a total of 6 batches.

I then used the noted absorbance values from the standards and their known Catechin concentrations to calculate a linear standard curve in Excel. Phenolic concentration values were then calculated as:

$$P_i = b \times a_i + m \quad (1)$$

Where P_i is phenolic content for tree i(mg phenolics/gram dry litter); b is the slope of the calculated trendline; a_i is the absorption value for tree i; m is the value where the trendline intersects the y-axis when the absorption value is 0.

2.3.2. Trait calculations and data preparation

As a basis for further trait measurements and calculations I used the previously collected samples as well as the dataset of previously measured traits of the samples. Calculations of traits were performed in MS Excel and R (R Core Team, 2019). I calculated the trait Specific Leaf Area (SLA) as:

$$SLA_i = \frac{SA_i}{NW_i \times 1000} \quad (2)$$

with SLA_i being specific leaf area for tree i (mm^2/mg); SA_i is the average surface area for tree i; NW_i is the average needle weight for tree i multiplied with 1000 to get the weight in milligrams. I calculated averages for height, width and weight of all sampled cones per tree. For these calculations I ignored classifications of mature or immature for each cone.

Prior to statistical analyses, I examined the dataset of the measured and calculated trait variables for the 320 sampled trees to identify extreme values. For each trait variable I defined extreme values as either: values smaller than the first quartile value subtracted with three times the interquartile range value between quartile 1 and 3, or values larger than the third quartile value added with three times the interquartile range value between quartile 1 and 3 (Prins et al. 2012). Such extreme values were likely due to measurement or data entry errors, and were therefore removed prior to further data treatment and analyses. To enable an evaluation of possible differences in traits between subspecies, a mean site value was calculated for each site and trait variable. The site mean value was based on the values of the 8 sampled trees at each site. I also used the standardized measure: coefficient of variation (CV) to examine possible differences in trait variation. CV was calculated for each trait and site as:

$$CV_i = \frac{\sigma_i}{\mu_i} \quad (3)$$

Where CV_i is the coefficient of variation ratio of the studied trait variable for site i; σ_i is the standard deviation for the studied trait variable based on the 8 trees at site i; μ_i is the mean value for the studied trait variable based on the 8 trees at site i. I used the CV value to represent the variation of the studied trait variable between the 8 trees sampled at each site.

2.4. Statistical analyses

For the statistical analyses I used: RStudio (R Core Team, 2019) for Univariate Statistical analyses and Primer package version 7 (Clarke & Gorley, 2015) for multivariate data analyses. In the analyses I used 22 trait variables. I created figures in R, MS Excel and Powerpoint.

All analyses on individual sites were considered to be the unit of replication, which includes 8 replicates for each of the North American subspecies, and 8 paired replicates for each of the Patagonia Lodgepole pine types (plantation or invasion front). To test if there was a difference in traits between the three native subspecies and Patagonia Lodgepole pine plantations, I used non-parametric multivariate analyses of variances (PERMANOVA) with Bray-Curtis distances applied to the data set (Anderson, 2001). One PERMANOVA was performed on the mean trait values, and a second PERMANOVA was performed on the mean coefficient of variance values, with each of the four tree types serving as fixed factors (i.e. subspecies *contorta, latifolia* and *murrayana,* and Patagonia *P. contorta* plantations). To test for differences in traits of the introduced plantation trees and the invasive trees, I performed PERMANOVAs for mean trait and CV values as described above; however, because of the paired sampling design in Patagonia, I also included site as a random factor in these analyses. The paired sampling design between the two Patagonian categories was also the cause to why only Patagonian plantations were compared against the native subspecies. To graphically display PERMANOVA analysis of mean trait values and mean CV values, I performed two Canonical Analysis of Principal coordinates (CAP), one for mean trait values, and another for mean CV values. These CAP analyses included all five *P. contorta* categories (Native subspecies *contorta, latifolia, murrayana,* and Patagonian plantations and invasion fronts), which allowed visualization of all categories relative to one another based on their mean trait or CV values.

When PERMANOVA tests indicated significant differences, I performed post-hoc univariate analyses to determine which specific trait variables were significantly different (Figure 3). This included one-way ANOVAs for each trait across the four *P. contorta* categories (i.e. subspecies *Contorta, Latifolia* and *Murrayana,* and Patagonia *P. contorta* plantations), and paired *t*-tests for each variable between the two Patagonia *P. contorta* tree types (i.e. plantation or invaders). For all univariate analysis, I tested for assumption of normality using Shapiro-Wilk tests on the residuals of a linear model of the data, and Levene's tests for the assumption of homogeneity of variance. If the data did not meet the assumptions, either a log- or BoxCox-transformation was done. When assumptions were met, one-way ANOVA or *t*-tests were performed. When data could not be transformed to meet parametric assumptions, Welch´s One way ANOVA tests or Kruskal-

Wallis tests were used. If there was a difference between the groups, further post-hoc pairwise analyses were performed to examine which group differed from which. ANOVAs were followed by Tukey´s post-hoc tests, Welch's One Way ANOVAs were followed by Games-Howell post-hoc tests, and Kruskal Wallis tests were followed using Dunn´s post-hoc tests.

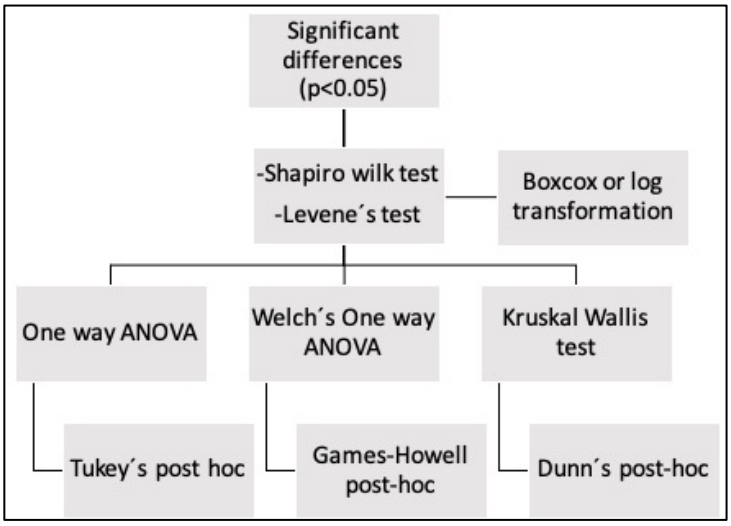


Figure 3. Description of workflow if PERMANOVA indicated significant differences(p-value < 0.05) in mean trait values or CV values between the native subspecies and Patagonian Pinus contorta *plantations.*

Tests for detecting differences in trait values and trait variability (means and coefficient of variation) between the plantations and the invasion front in Patagonia were performed with a paired t-test or the non-parametric Wilcoxon signed-rank tests (Figure 4). Plantation values and invasion front values were paired with site as the pairing factor. Assumption of normality of the differences between the paired observations was tested with Shapiro-Wilk test. Data were log-transformed if the normality assumption was not met. A paired t-test was performed on the trait variables that met the normality assumption. For the trait variables that did not meet the normality assumption, even when transformed, I instead applied the non-parametric Wilcoxon signed-rank test. For all performed statistical analyses I have considered a p-value < 0.05 as evidence of significance.

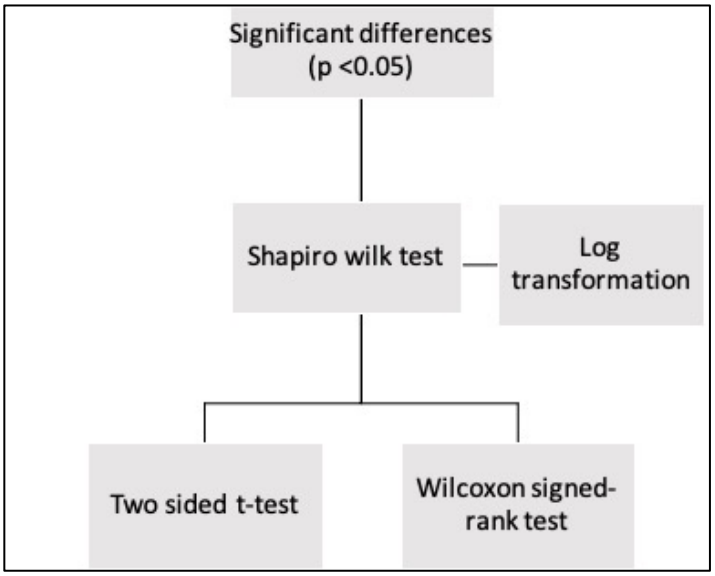


Figure 4. Description of workflow if PERMANOVA indicated significant differences(p-value < 0.05) in mean trait values or CV values between the plantations and invasion fronts trees of Pinus contorta *in Patagonia .*

Boxplots for all five categories were created to display the results of the pairwise comparisons on mean and CV values of the four *P. contorta* categories and the pairwise comparison between Patagonian *P. contorta* plantations and invasion fronts.

3. Results

3.1. Mean trait values

Among the four *P. contorta* categories (native subspecies *contorta, latifolia* and *murrayana,* and Patagonia *P. contorta* plantations) the non-parametric multivariate analysis of variances (PERMANOVA) indicated a significant difference ($p < 0.05$) in mean trait values between the categories (Table 1). The PERMANOVA found the native *contorta* to be the native subspecies least similar to the Patagonian plantations (PP) which can also be seen in the CAP diagram which graphically displays the results found by the PERMANOVAs (Figure 5). PERMANOVA pairwise comparisons indicated *murrayana* to be the native subspecies with the least trait differences to PP, even though *latifolia* also was indicated to be almost as similar. Displayed in the CAP diagram are also the relative high similarities found by the PERMANOVA in traits of native subspecies *latifolia* and *murrayana,* as they are displayed more clustered to each other relative to PP and *contorta*. The two Patagonian categories, invasion front and plantations, are displayed overlapping and clustered together, indicating a strong association of the data between the categories, even though the Patagonian invasion (PI) category was not included in the PERMANOVA with the other four categories.

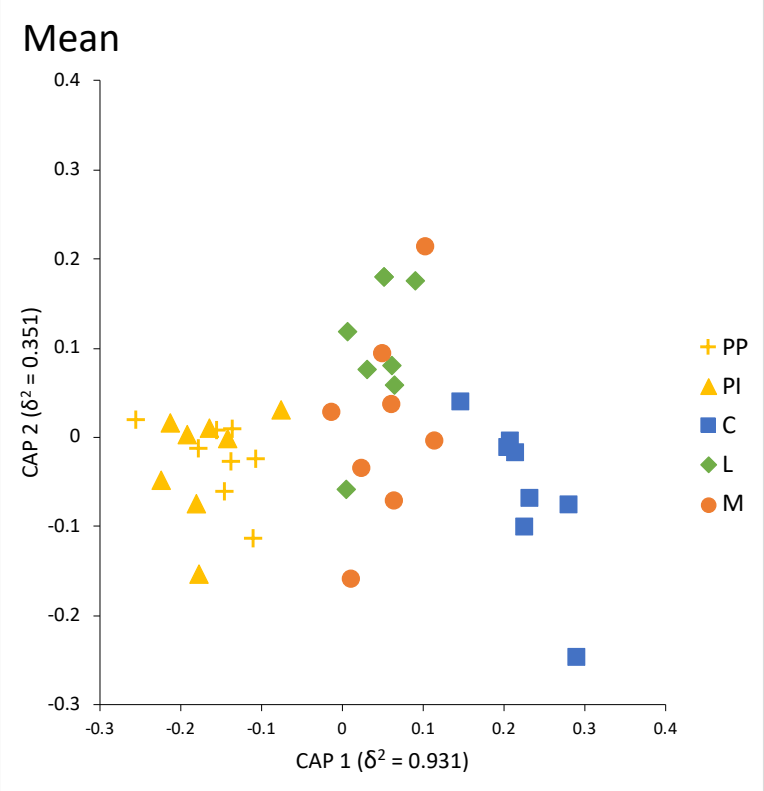


Figure 5. Results of Canonical Analysis of Principal coordinates on mean trait values for sites of the five Pinus contorta categories. PP represents Patagonian plantation sites, PI represents Patagonian invasion front sites, C represents sites with native subspecies contorta, L represents sites with native subspecies latifolia and M represents sites with native subspecies murrayana. δ^2 values on the axes signalize the strength of the association between the data cloud of mean trait values and the hypothesis of differences between the five categories.

Follow up univariate post-hoc analyses showed that 21 of the 22 tested trait variables were significantly different between the categories (Table 1). Phosphorus content was the only trait variable that did not show a significant difference between the categories. Needle weight had the highest F-value (89.72) out of the significant trait variables.

Table 1. Results of multivariate and univariate analyses of mean trait values for subspecies contorta, latifolia and murrayana*, and Patagonia* Pinus contorta *plantations.* indicates ANOVA was performed on transformed values.[a] represents a Pseudo-F-value, [b] represents F-value, [c] represents a chi-square value. Significant p-values are in bold (p-value < 0.05). SLA stands for specific leaf area.*

Means		***Analyse method***	***Test statistic value***	***p-value***
	Main categories			
	Native subspecies and the Patagonian plantation subspecies	PERMANOVA	15.404[a]	**0.001**

	TRAITS			
Needles				
	Weight	ANOVA	89.72[b]	**<0.001**
	Length	ANOVA*	18.3[b]	**<0.001**
	Projected area	ANOVA*	66.21[b]	**<0.001**
	Surface area	ANOVA*	66.21[b]	**<0.001**
	Carbon content	ANOVA	8.882[b]	**<0.001**
	Cellulose content	Welch´s One way ANOVA	33.964[b]	**<0.001**
	Lignin content	ANOVA	23.57[b]	**<0.001**
	Nitrogen content	ANOVA	3.612[b]	**0.0254**
	Phosphorus content	ANOVA	2.131[b]	0.119
	Phenolic content	ANOVA	14.73[b]	**<0.001**
	SLA	ANOVA	23.88[b]	**<0.001**
Seeds				
	Weight	Welch´s One Way ANOVA	15.595[b]	**<0.001**
	Length	Kruskal-Wallis rank sum test	10.629[c]	**0.014**
	Projected area	ANOVA*	8.142[b]	**<0.001**
	Surface area	ANOVA*	8.333[b]	**<0.001**
Cones				
	Mature cone width	ANOVA	9.757[b]	**<0.001**
	Mature cone length	ANOVA	8.47[b]	**<0.001**
	Mature cone weight	ANOVA	29.77[b]	**<0.001**
	Immature cone weight	ANOVA*	26.06[b]	**<0.001**
	Cone width	ANOVA	15.95[b]	**<0.001**
	Cone length	ANOVA	7.748[b]	**<0.001**
	Cone weight	ANOVA*	41.98[b]	**<0.001**

The pairwise comparisons after the univariate analyses on mean values of the four categories showed that the native subspecies *contorta* was significantly different from the other two native subspecies for 7 variables, all connected to needle traits (Figure 6). In total, *contorta* was significantly different from *murrayana* for 10 trait variables and from *latifolia* for 7 variables. The native subspecies *latifolia* and *murrayana* were only significantly different from each other regarding one trait variable, which was cellulose content. The Patagonian plantation category was different from all three native subspecies for 9 trait variables, with for example a higher phenolic content, lower SLA and heavier cones (mature, immature and cones overall). Overall the traits of the Patagonian plantations were most different from the native *contorta,* with 19 traits being significantly

different, supporting the pattern shown in the CAP diagram where *contorta* sites were furthest away from Patagonian plantation sites (Figure 5). Comparing Patagonian plantation to the category *latifolia* showed a significant difference in 18 of the displayed trait variables. Patagonian plantation and *murrayana* had 10 significantly different trait variables.

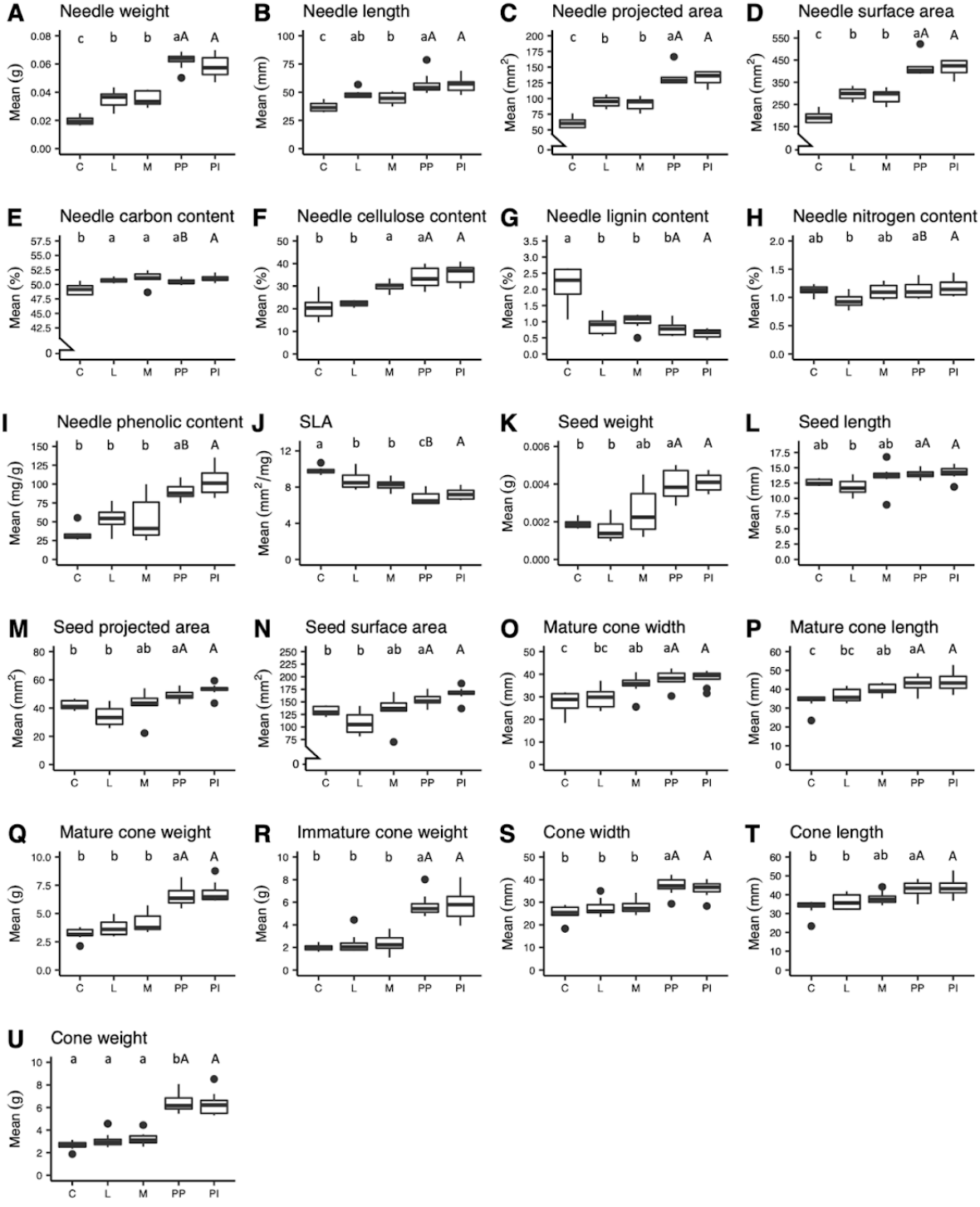


Figure 6. Trait variables with significant difference (p value < 0.05 from univariate analyses) between categories based on mean values of sites. The letter C represents the box for native subspecies contorta*, L represents the box for native subspecies* latifolia*, M represents the box for native subspecies* murrayana*, PP represents the box for Patagonian* P. contorta *plantation, PI represents the box for Patagonian* Pinus contorta *invasion front. Lower case letters display results of pairwise comparison between the first four categories and uppercase letters display the result of the pairwise comparison between the two Patagonian categories. A shared letter indicates no significant difference between the compared categories. SLA stands for specific leaf area.*

Mean trait values of the two Patagonian Lodgepole pine categories, invasion front and plantation, differed significantly from each other, as revealed by the PERMANOVA, even though they are displayed as relatively tightly clustered by the CAP analysis (Table 2: Figure 5). From the post-hoc analyses with paired t-tests and Wilcoxon signed rank it was shown that the two categories were significantly different for Carbon content, Nitrogen content, Phenolic content and SLA. Generally the invasion front trees showed higher values for these variables than the plantation trees (Figure 6).

Table 2. Results of multivariate and univariate analyses of mean trait values for Patagonia Pinus contorta *plantations and Patagonia P. contorta invasion fronts.* [a] *represents a Pseudo-F-value,* [b] *represents an absolute t-value,* [c] *represents a v-value. Significant p-values are in bold (p-value<0.05). SLA stands for specific leaf area.*

Means		***Analysis method***	***Test statistic value***	***p-value***
	MAIN CATEGORIES			
	Plantation vs Invasive in Patagonia	PERMANOVA	3.144[a]	**0.005**
	TRAITS			
Needles				
	Weight	Paired t-test	1.341[b]	0.222
	Length	Paired t-test	0.238[b]	0.819
	Projected area	Paired t-test	0.016[b]	0.988
	Surface area	Paired t-test	0.095[b]	0.927
	Carbon content	Wilcoxon signed rank test	33[c]	**0.039**
	Cellulose content	Paired t-test	1.610[b]	0.151
	Lignin content	Paired t-test	1.892[b]	0.100
	Nitrogen content	Paired t-test	2.446[b]	**0.044**
	Phosphorus content	Paired t-test	0.152[b]	0.883
	Phenolic content	Paired t-test	4.035[b]	**0.005**
	SLA	Wilcoxon signed rank test	36[c]	**0.008**
Seeds				
	Weight	Paired t-test	0.343[b]	0.742
	Length	Paired t-test	0.380[b]	0.716
	Projected area	Paired t-test	1.438[b]	0.194
	Surface area	Paired t-test	1.438[b]	0.194
Cones				
	Mature cone width	Paired t-test	0.244[b]	0.814
	Mature cone length	Paired t-test	0.569[b]	0.587
	Mature cone weight	Paired t-test	0.585[b]	0.577
	Immature cone weight	Paired t-test	0.132[b]	0.899
	Cone width	Paired t-test	1.166[b]	0.282

	Cone length	Paired t-test	0.554[b]	0.597
	Cone weight	Paired t-test	0.109[b]	0.916

3.2. Trait variation values

A significant difference in trait CV values between the four categories was revealed by the PERMANOVA (Table 3). A difference between the categories can also be seen in the CAP diagram based on the pattern of clustering of the observations for each category (Figure 7). Pairwise comparisons from the PERMANOVA on CV values indicated all four categories except for *latifolia* and *murrayana* to be different from each other in the variation of traits (Figure 5). The two Patagonian categories (though PI was not included in the PERMANOVA with native subspecies) appear relatively similar in trait variation compared to the other categories, as they are displayed as fairly tightly clustered.

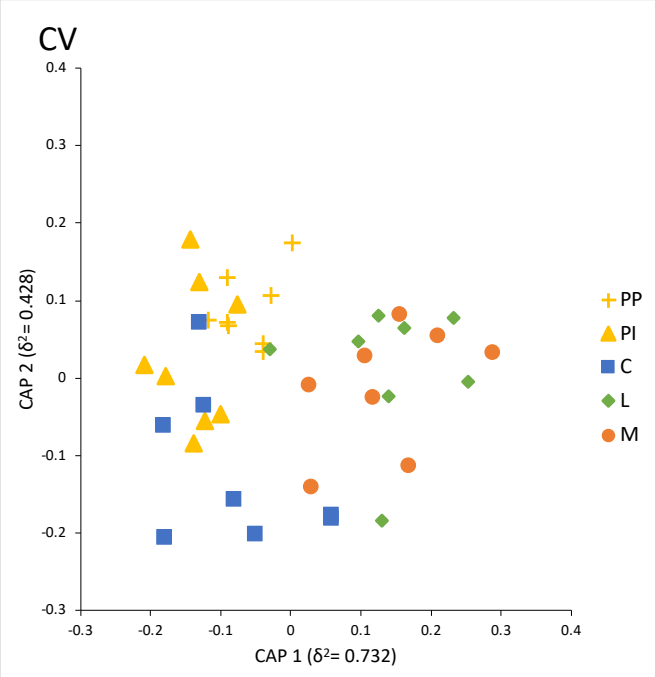


Figure 7. Results of the CAP analysis on coefficient of variation (CV) values for sites of the five Pinus contorta *categories. PP represents Patagonian plantation sites, PI represents Patagonian invasion front sites and C, L and M represents sites with native subspecies* contorta, latifolia *and* murrayana*, respectively. δ^2 values on the axes signalize the strength of the association between the data cloud of CV values and the hypothesis of differences between the five categories.*

Of the 22 trait variables, 12 showed a significant difference in trait variation between the categories as shown by the post-hoc analyses (Table 3).

Needles, seeds and cones all had some trait variables that between the categories were significantly different. The number of trait variables with a significant difference based on CV values were fewer compared to the analyses based on mean values (Table 1). A significant difference between the four categories was discovered for the traits: Needle length, Needle projected area, Needle surface area, Needle cellulose content, Needle phenolic content, Seed projected area, Seed surface area, Mature cone width, Immature cone weight, Cone width, Cone length and Cone weight (Table 3).

Table 3. Results of the multivariate and univariate analyses of coefficient of variation values for subspecies contorta*,* latifolia *and* murrayana*, and Patagonia* Pinus contorta *plantations. * indicates ANOVA was performed on transformed values.[a] represents a Pseudo-F-value, [b] represents F-value, [c] represents a chi-square value. Significant p-values are in bold (p-value < 0.05). SLA stands for specific leaf area.*

Coefficient of variation		***Analysis method***	***Test statistic value***	***p-value***
	MAIN CATEGORIES			
	Native subspecies and the Patagonian plantation subspecies	PERMANOVA	3.266[a]	**0.001**
	TRAITS			
Needles				
	Weight	ANOVA*	2.054[b]	0.129
	Length	ANOVA	3.136[b]	**0.041**
	Projected area	ANOVA	3.932[b]	**0.019**
	Surface area	ANOVA	3.932[b]	**0.019**
	Carbon content	ANOVA*	2.021[b]	0.134
	Cellulose content	ANOVA*	6.992[b]	**0.001**
	Lignin content	ANOVA*	0.375[b]	0.772
	Nitrogen content	ANOVA*	1.006[b]	0.405
	Phosphorus content	ANOVA	1.067[b]	0.379
	Phenolic content	ANOVA*	4.021[b]	**0.017**
	SLA	Kruskal-Wallis rank sum test	3.5597[c]	0.313
Seeds				
	Weight	ANOVA	2.889[b]	0.056
	Length	ANOVA	1.833[b]	0.168
	Projected area	ANOVA*	5.746[b]	**0.004**
	Surface area	ANOVA*	5.978[b]	**0.003**
Cones				
	Mature cone width	ANOVA*	3.242[b]	**0.037**
	Mature cone length	ANOVA*	2.685[b]	0.066
	Mature cone weight	Kruskal-Wallis rank sum test	7.543[c]	0.056

	Immature cone weight	ANOVA*	4.983[b]	**0.007**
	Cone width	Kruskal-Wallis rank sum test	16.707[c]	**<0.001**
	Cone length	ANOVA*	2.963	**0.049**
	Cone weight	ANOVA*	3.54	**0.027**

Pairwise comparisons, after the univariate analyses on CV values for the four categories, showed that the category native subspecies *contorta* differed significantly from *latifolia* and *murrayana* for 5 trait variables each (Figure 8). For those variables *contorta* generally displayed higher CV values. *Murrayana* had significantly lower CV values than *latifolia* for seed projected area and seed surface area, which also were the only two trait variables with a significant difference between the two categories. Based on the boxplot display, Patagonian plantations visually appear to have lower CV values compared to the native subspecies; however, this was not supported by the post-hoc pairwise analysis, since the Patagonian plantation category was not significantly different from at least one other category or more for all of the studied trait variables. The Patagonia plantation category statistically differed from *contorta* for 5 trait variables, *latifolia* for 2 trait variables and from *murrayana* for 3 trait variables.

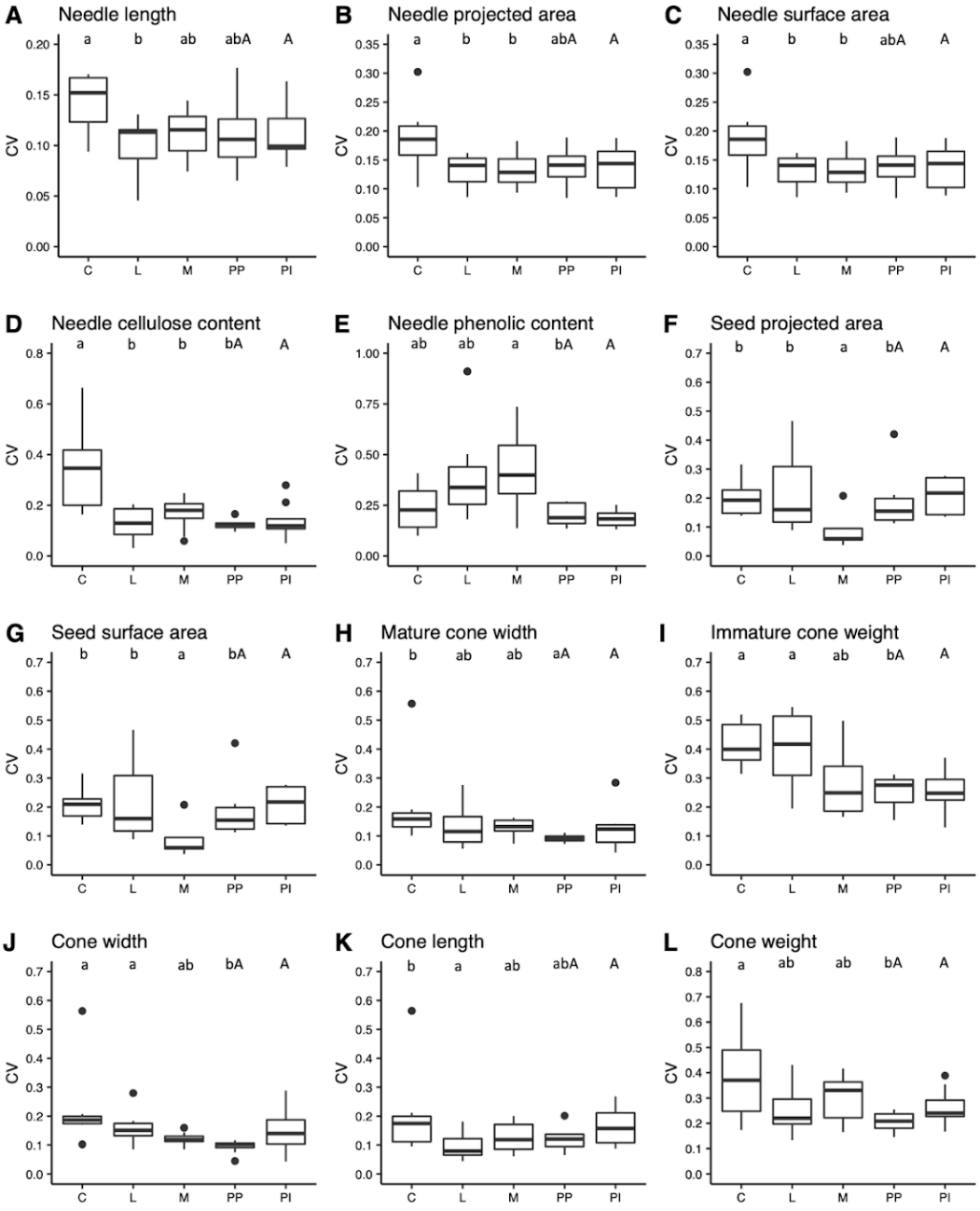


Figure 8. Trait variables with significant differences (p value < 0.05 from univariate analyses) between categories based on CV values for sites. The letter C represents the box for native subspecies contorta*, L represents the box for native subspecies* latifolia*, M represents the box for native subspecies* murrayana*, PP represents the box for Patagonian* Pinus contorta *plantation, PI represents the box for Patagonian* P. contorta *invasion front. Lower case letters display results of pairwise comparison between the first four categories and uppercase letters display the results of the pairwise comparison between the two Patagonian categories. A shared letter indicates no significant difference between the compared categories. SLA stands for specific leaf area.*

No significant difference was observed using the multivariate analysis (i.e. PERMANOVA) of CV values between the two Patagonian categories Patagonian plantation and Patagonian Invasion front (Table 4). None of the following post-hoc analyses showed a significant difference between the two categories for any trait variable, supporting the result of the PERMANOVA.

Table 4. Results of multivariate analysis and univariate analysis of coefficient of variation values for Patagonia Pinus contorta *plantations and Patagonia* P. contorta *invasion fronts. * indicates t-test was performed on transformed values [a] represents a Pseudo-F-value, [b] represents an absolute t-value. Significant p-values are in bold(p-value<0.05). SLA stands for specific leaf area.*

Coefficient of variation		***Analysis method***	***Test statistic value***	***p-value***
	MAIN CATEGORIES			
	Plantation vs Invasive in Patagonia	PERMANOVA	1.076[a]	0.401
	TRAITS			
Needles				
	Weight	Paired t-test	1.406[b]	0.203
	Length	Paired t-test	0.188[b]	0.856
	Projected area	Paired t-test	0.126[b]	0.903
	Surface area	Paired t-test	0.093[b]	0.929
	Carbon content	Paired t-test*	1.275[b]	0.243
	Cellulose content	Paired t-test	0.438[b]	0.675
	Lignin content	Paired t-test	1.736[b]	0.126
	Nitrogen content	Paired t-test	0.739[b]	0.484
	Phosphorus content	Paired t-test	0.547[b]	0.602
	Phenolic content	Paired t-test	0.489[b]	0.64
	SLA	Paired t-test	1.770[b]	0.120
Seeds				
	Weight	Paired t-test	0.704[b]	0.504
	Length	Paired t-test	1.465[b]	0.186
	Projected area	Paired t-test	0.653[b]	0.535
	Surface area	Paired t-test	0.653[b]	0.535
Cones				
	Mature cone width	Paired t-test	1.116[b]	0.301
	Mature cone length	Paired t-test	1.973[b]	0.089
	Mature cone weight	Paired t-test	1.849[b]	0.107
	Immature cone weight	Paired t-test	0.160[b]	0.877
	Cone width	Paired t-test	1.756[b]	0.123
	Cone length	Paired t-test	1.761[b]	0.122
	Cone weight	Paired t-test	1.774[b]	0.119

4. Discussion

4.1. Native subspecies

For this thesis, I hypothesized that the three subspecies would have significant trait differences within their native range. Regarding needle traits, I found significant differences between subspecies *contorta* compared to subspecies *latifolia* and *murrayana* (Figure 6). In comparison, the needles for subspecies *contorta* were lighter, shorter, had a smaller projected area and surface area, a lower carbon content, a higher lignin content and also the highest SLA. Previous descriptions of the needles of subspecies *contorta,* described the needles to be short and relatively narrow compared to *latifolia* and *murrayana* (Lotan & Critchfield, 1990; Critchfield, 1957), which my data support. Also my observation of generally smaller projected areas and surface areas is consistent with these previous studies, since the two variables are based on measurements of needle length and width. Any significant differences in needle traits between *latifolia* and *murrayana* could only be detected for cellulose content, contradicting previous descriptions of the subspecies *latifolia* having long and relatively wide needles and the *murrayana* generally having medium short and very wide needles (Lotan & Critchfield, 1990). According to Wheeler et al. (1983) there are also differences in seed size between the subspecies, which in this study could not be distinguished statistically. For mature cones the results show that *murrayana* has significantly longer and wider cones than *contorta*, which corresponds with cone descriptions of Wheeler et al. (1983) and Koch (1996). I could not distinguish the same pattern for cones overall and I found no significant difference in weight between either of the subspecies for the three cone classifications even though a weak visual pattern of heavier cones for *murrayana* can be detected from the boxplots. Previous descriptions of cone and seed traits of the different subspecies sometimes contradict each other which implies that such traits of the subspecies can vary (Lotan & Critchfield, 1990: Critchfield, 1957: Wheeler et al. 1983:Koch, 1996). Therefore, I believe cone traits might not offer the best basis for distinguishing the subspecies from each other, since there appears to be a lot of within subspecies variation in cone traits.

Overall, the finding that several trait variables differed significantly when comparing *contorta* relative to the other native subspecies supports my hypothesis of trait differences between the native subspecies. Contradictory to my hypothesis is the result that only one trait variable was significantly different between the subspecies *murrayana* and *latifolia*. A possible explanation could be the mixing

of the two subspecies in the area we sampled, which was described by Lotan & Critchfield (1990). Since the subspecies are also known to be inter-fertile, another possible explanation could be that the samples used for analyses in this thesis actually came from hybrids of the subspecies. Hence, it appears that there are some trait differences between the three native subspecies examined in this thesis but the pattern between at least two of the subspecies was not as strong as I expected.

4.2. Patagonian *P. contorta* in comparison to native subspecies

The second hypothesis for this thesis was regarding expectations of the Patagonian *P. contorta* traits to be most similar to the subspecies *murrayana.* The PERMANOVA analysis on mean trait values partly supports this hypothesis as the site observations of the Patagonian *P. contorta* plantations (PP) observations were the least similar to the *contorta* observations (Figure 5). It also indicated PP was somewhat more similar to *murrayana* than *latifolia.* Despite the similarities to the native subspecies, we also observed clear differences between all four categories. The results of the univariate analyses and pairwise comparisons supported my first interpretations of the PERMANOVA visualized in the CAP diagram, with PP having the least significant trait differences to *murrayana* (Figure 6). Thus even though Langdon et al. (2010) states that *latifolia* may have also been commonly introduced to the region, my results however suggest *murrayana* is most likely the main subspecies introduced to Patagonia, at least at the sites sampled for this study.

My third hypothesis predicted that introduced *P. contorta* in Patagonia would display trait values that were different from the three native subspecies. In support of this hypothesis, PP had several traits that were significantly different from all the native subspecies (Figure 6). Possible explanation for these differences could be phenotypic plasticity to differences in the environment in the two regions (Fitter & Hay, 2002). It is well known that environmental factors, such as climate or soils, influence plant growth and physiology (E), and thus also their traits. Patagonian *P. contorta* might therefore experience other limitations and availability of resources than the native subspecies in the Pacific northwest which in turn resulted in differences in the measured traits. *P. contorta* is also a species often associated with invasions (Ledgard, 2001: Langdon et al. 2010) and invasive species often have a high phenotypic plasticity (Davidson et al. 2001). The noted differences in traits could thus be the result of a high ability of the genotypes to respond to the experienced environment in Patagonia and influence the phenotype, basically a combination of genetic and environmental influence (G x E). Another explanation to the difference in traits could be due to only genetic influence (G), i.e., genetic differences between the native subspecies and PP, due to founder effects (i.e., the founding population had uncommon trait values). All the above-mentioned ideas could be applied to explain and be supported by my results.

For my fourth hypothesis, I expected the introduced *P. contorta* population to have a lower variability of traits than the native subspecies (due to a population bottleneck), which I evaluated by examining traits coefficients of variation (CVs). Based on the pairwise comparisons I found that the CVs of the PP group overall did not significantly differ from the native subspecies (Figure 8). For each studied trait variable, PP was never statistically different from all of the native subspecies. These results stand in contrast to the stated hypothesis, even though it appears from the boxplots that the PP category often trends towards lower trait variation compared to the native subspecies. Warwick et al. (1987) also compared variation of traits but in grass of native and introduced *Apera spica-venti.* In their study, they similarly found that the variation of most traits did not significantly differ between native and introduced groups, and for the traits where the variation did differ significantly, that there was no consistent pattern of the introductions always having lower variation. Additionally Dlugosch & Parker (2008) reviewed previously published literature and came to the conclusion that invasions generally do not display a lower variation in traits controlled by multiple genes compared to their native populations even though they often experience losses of genetic diversity, which could support my results. Analyses of CV values in this thesis thus indicate that although the Patagonian *P. contorta* might have experienced a genetic bottleneck when introduced to Patagonia, it appears as the variation of traits are not significantly affected by this.

For my fifth hypothesis, I expected to find differences in traits between plantations and invasion fronts of the Patagonian *P. contorta*. Plantation trees represent the first generation of *P. contorta* in Patagonia, while the trees at the invasion front (i.e. the *P. contorta* trees located furthest away from the plantations) most likely are of later generations. I found significant trait differences in four traits between the PP and PI (Table 2: Figure 6). As concluded by Whitney & Gabler (2008) a fast evolution often occurs in traits frequently associated with invasion success of invading species which could offer an explanation to the noted differences. They mention fast growth to be a trait associated with successful invasion, where fast evolution has been known to occur. Since a high SLA is often associated with high growth rates (Poorter & Remkes, 1990), SLA is thus a representative growth trait. Therefore, the significantly higher SLA of PI compared to PP I observed is consistent with this pattern, and might suggest support for the EICA hypothesis (i.e. rapid evolution of growth instead of defense). However, the EICA hypothesis also suggests that enemy release should result in rapid evolution of growth versus defense traits. Based on these hypotheses as described by Mitchell & Power (2003) and Bossdorf et al. (2005) one would expect PI to have invested less resources into defense traits and more into growth traits compared to the PP and the native subspecies, if the introduced population experienced a release of enemies in Patagonia compared to the native range. Foliar phenolics are often associated with plant defense against enemies (Lattanzio et al. 2006) and thus represent a defense trait. My findings of PI having higher phenolic values than PP, and PP in turn having significantly higher values than the native subspecies, is thus not

compatible with one of the main predictions of the EICA hypothesis, i.e. that defense should decline in introduced and invading plant populations. In addition I also found an overall lower SLA in Patagonia, as PP has significantly lower SLA levels than the native subspecies (Figure 6). Poorter (1989) stated that selection on plants growing in poor environments often favors traits that ensure long leaf survival, such as a build up of phenolics, but these traits are often also associated with a lower SLA. The noted trends of phenolics and SLA might therefore also be the result of a selection formed by a less favorable environment in Patagonia compared to the native range. What needs to be considered when discussing selection and hypotheses connected to evolution though, is that trees are long-lived and slow-growing compared to many other plants, i.e grasses. Most likely there are thus only a few generations between the trees in the plantation and the trees at the invasion fronts. The invasion front trees might even stem from long-dispersed seeds of the plantation trees, resulting in a difference of only one generation. When comparing PI and PP there are therefore probably not many generations between them, consequently giving little time for evolutionary processes. It is thus difficult to believe evolution alone could explain the noted trait differences.

Another explanation to the observed trait differences between PP and PI is instead connected to the age of the trees. The plantation trees are older than the invasion front trees, since the latter stem from the plantation trees. It is known that ontogeny can influence traits and Barton & Koricheva (2010) for example found that trees in the seedling stage often had higher levels of substances associated with plant defense than trees in later life stages. Environmental dissimilarities between the plantations and the invasion fronts might likewise offer an explanation for trait differences. The PI and PP trees are experiencing differences in their environments (i.e., inter and intraspecific competition), and these contrasts might cause trait differentiation. Overall, the difference in age between the two studied Patagonian categories and the environmental differences could thus offer potential explanations, possibly even more credible than evolution, for the noted trait differences.

4.3. Conclusion

Based on the results presented in this thesis I conclude that there are some trait differences between the three subspecies of *P. contorta*, *contorta, latifolia* and *murrayana* within their native range. *Contorta* is the most different from the other two native subspecies and I found almost no difference between the subspecies *latifolia* and *murrayana* since they were only significantly different from each other for one of all of the studied traits. The mean trait values of the Patagonian *P. contorta* were most similar to the native subspecies *murrayana,* thus suggesting that *murrayana* is the main subspecies introduced to Patagonia, in support for my hypotheses. At the same time, there are also obvious trait differences between the

native subspecies and the Patagonian *P. contorta.* This implies that *P. contorta* has experienced some sort of genetic or phenotypic mechanism causing its traits to differentiate from other native subspecies. It is difficult though to identify any one particular mechanism that could have acted alone, or in combination with others in both time and space, without performing detailed genetic analysis of the populations. I also found no evidence of lower trait variation in the introduced range compared to the native range of *P. contorta*, which suggests genetic bottlenecks during introduction have not constrained traits in the new range. There are some trait differences between plantation and invasion front trees of Patagonian *P. contorta,* which may suggest some rapid evolution (i.e. change in gene frequency) may have occurred during invasion. However, these differences might also be the result of ontogenic or environmental differences between the plantations and invasions fronts.

Overall these results provide an example of how an invasive species can vary in traits. What needs to be considered though is that I have only compared Patagonian *P. contorta* to the native range, which can be considered as a weakness in the study. Since *P. contorta* has also been introduced to many other parts of the world (Ledgard, 2001: Langdon et al. 2010), such as New Zealand and Northern Europe, there is a need and opportunity for further research to determine if the patterns described in this study can be seen in other places as well. It could also be interesting to further investigate the environmental differences between the native and invasive range to better understand their potential influence on traits. By investigating these above mentioned suggestions, we may be able to further increase our understanding of *P. contorta* invasion, and what enables it and other species to become successful invaders. Additional research on the combination of genetics and traits of invasive species could also aid in specifying what specific mechanisms influence traits and their impact on successful invasions.

References

Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* 26(1), 32-46. DOI: https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Barker; H.L., Holeski, L.M. & Lindroth, R.L. 2018. Independent and interactive effects of plant genotype and environment on plant traits and insect herbivore performance: A meta-analysis with Salicaceae. *Functional Ecology,* 33(3), 422-435. DOI: https://doi.org/10.1111/1365-2435.13249

Barton, K.E., Koricheva, J. 2010. The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. *The American Naturalist,* 175(4), 481-493. DOI: https://doi.org/10.1086/650722

Blossey, B. & Nötzold, R. 1995. Evolution of increased competitive ability in invasive nonindigeneous plants: a hypothesis. *Journal of Ecology,* 83(5), 887-889. DOI: https://doi.org/10.2307/2261425

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. 2005. Phenotypic and genetic differentiation between native an introduced plant populations. *Oecologia*, 144, 1-11. DOI: 10.1007/s00442-005-0070-z

Bradshaw, A.D. 1965. Evolutionary Significance of Phenotypic Plasticity in Plants. *Advances in Genetics,*13, 115-155. DOI: https://doi.org/10.1016/S0065-2660(08)60048-6

Clarke, K.R. & Gorley, R.N. 2015. *PRIMER v7: User Manual/Tutorial* (7). [Software] http://updates.primer-e.com/primer7/manuals/User_manual_v7a.pdf [2021-02-01]

Critchfield, W.B. 1957. *Geographic variation in Pinus contorta.* Cambridge, Mass.

Davidson, A.M., Jennions, M. & Nicotra, A.B. 2001. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters,* 14, 419-431. DOI: https://doi.org/10.1111/j.1461-0248.2011.01596.x

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., Prentice, I.C., Garnier, E., Bönisch, G., Westoby, M., Poorter, H., Reich, P.B., Moles, A.T., Dickie, J., Gillison, A.N., Zanne, A.E., Chave, J., Wright, S.J., Sheremet'ev, S.N., Jactel, H.,

Baraloto, C., Cerabolini, B., Pierce, S., Shipley, B., Kirkup, D., Casanoves, F., Joswig, J.S., Günther, A., Falczuk, V., Rüger, N., Mahecha, M.D. & Gorné, L.D. 2016. The global spectrum of plant form and function. *Nature*, 529**,** 167–171. DOI: https://doi.org/10.1038/nature16489

Dlugosch, K.M. & Parker, I. M. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology,* 17(1), 431-449. DOI: https://doi.org/10.1111/j.1365-294X.2007.03538.x

Drenovsky, R. E., Grewell, B.J., D'Antonio , C.M., Funk, J.L., James, J.J., Molinari, N., Parker, I.M. & Richards, C.L. 2012. A functional trait perspective on plant invasion *Annals of Botany,* 110(1), 141-153. DOI: https://doi.org/10.1093/aob/mcs100

Ehrenfeld, J.G. 2003. Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. *Ecosystems,* 6, 503-523. DOI: https://doi.org/10.1007/s10021-002-0151-3

Fitter, A.H. & Hay, R.K.M. 2002. *Environmental Physiology of Plants.* Third edition. London: Academic Press.

Fritts, T.H. & Rodda, G.H. 1998. The role of introduced species in the degradation of island ecosystems: a case history of Guam. *Annual Review of Ecology and Systematics,* 29, 113-150. DOI: https://doi.org/10.1146/annurev.ecolsys.29.1.113

Geber, M.A. & Griffen, L.R. 2003. Inheritance and natural selection on functional traits. International Journal of Plant Sciences, 164(3), 21-42. DOI: https://doi.org/10.1086/368233

Gundale, M.J., Pauchard, A., Langdon, B., Peltzer, D.A., Maxwell, B.D. & Nuñez, M. A. 2014. Can model species be used to advance the field of invasion ecology? *Biological Invasions,* 16, 591-607. DOI: https://doi.org/10.1007/s10530-013-0610-0

Gurevitch, J., Fox, G.A., Wardle, G.M., Inderjit. & Taub, D. 2011. Emerging insinghts from the synthesis of conceptual framework for biological invasions. *Ecology letters,* 14(4), 407-418. DOI: https://doi.org/10.1111/j.1461-0248.2011.01594.x

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution,* 17(4), 164-170. DOI: 10.1016/S0169-5347(02)02499-0

Koch, P. 1996. *Lodgepole pine in North America. Lodgepole pine in North America.* Madison, Wis. : Forest Products Society.

Lake, J.C. & Leishman, M.R. 2004. Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from

herbivores. *Biological Conservation,* 117(2), 215-226. DOI: https://doi.org/10.1016/S0006-3207(03)00294-5

Langdon, B., Pauchard, A. & Aguayo, M. 2010. *Pinus Contorta* invasion in the Chilean Patagonia: local patterns in a global context. *Biological Invasions,* 12, 3961-3971. DOI: https://doi.org/10.1007/s10530-010-9817-5

Lattanzio, V., Lattanzio, V.M. & Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In: Imperato, F. (Ed.), Phytochemistry: Advances in Research. Trivandrum, India: Research Signpost. 23-67.

Ledgard, N. 2001. The spread of lodgepole pine (*Pinus contorta,* Dougl.) in New Zealand. *Forest Ecology and Management,* 141(1-2), 43-57. DOI: https://doi.org/10.1016/S0378-1127(00)00488-6

Lotan, J.E. & Critchfield, W. B. 1990. *Pinus contorta* Dougl. Ex Loud. Lodgepole Pine. Burns, R.M. & Honkala, B.H. (technical coordinators). *Silvics of North America: Volume 1. Conifers.* United States Department of Agriculture (USDA), Forest Service, Agriculture Handbook 654, 302-315.

Marbuah, G., Gren, I.M. & McKie, B. 2014. Economics of Harmful Invasive Species. A Review. *Diversity,* 6(3), 500-523. DOI: https://doi.org/10.3390/d6030500

Meyerson, L.A. & Mooney, H. A. 2007. Invasive species in an era of globalization. *Frontiers in Ecology and the Environment,* 5(4), 199-208. DOI: https://doi.org/10.1890/1540-9295

Mitchell, C. E. & Power, A. G. 2003. Release of invasive plants from fungal and viral pathogens. *Nature,* 421, 625-627. DOI: https://doi.org/10.1038/nature01317

National Geographic, Esri, Garmin, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, INCREMENT P Corp. 2020. *National Geographic World Map.* [Map] https://www.arcgis.com/home/item.html?id=b9b1b422198944fbbd5250b3241691b6 [2021-02-03]

Nei, M., Maruyama, T. & Chakraborty, R. 1975. The Bottleneck Effect and Genetic Variability in Populations. *Evolution,* 29(1), 1-10. https://www.jstor.org/stable/2407137?seq=3#metadata_info_tab_contents [2021-01-15]

Poorter, H. 1989. Interspecific variation in relative growth rate: On ecological causes and physiological consequences. I: Lambers, H., Cambridge, M.L., Konings, H. & Pons, T.L. (ed). *Causes and consequences of variation in growth rate and productivity of higher plants.* The Hague: SPB Academic Publishing. 45-68.

Poorter, H. & Remkes, C. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia,* 83, 553-559.

Prentis,P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. & Lowe, A.J. 2008. Adaptive evolution in invasive species. *Trends in Plant Science,* 13(6), 288-294. DOI: https://doi.org/10.1016/j.tplants.2008.03.004

Prins, J., McCormac, D., Michaelsson, D., Horrell, K. 2012. Products and Process Comparisons. In: Prins, J. *E- Handbook of Statistical Methods*. NIST/SEMATECH. DOI: https://doi.org/10.184334/M32189

R Core Team 2019. *R: A language and environment for statistical computing*. Vienna, Austria, R Foundation for Statistical Computing. [Software] https://www.R-project.org/ [2021-01-28]

Reich, P.B. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. *Journal of Ecology,* 102(2), 275-301. DOI: https://doi.org/10.1111/1365-2745.12211

Rejmánek, M. & Richardson D.M. 1996. What Attributes Makes Some Plant Species More Invasive? *Ecology,* 77(6), 1655-1661. DOI: 10.2307/2265768

Richards, C.L., Bossdorf, O., Norris, Z.M., Gurevitch, J. & Pigliucci, M. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters,* 9(8), 981- 993. DOI: https://doi.org/10.1111/j.1461-0248.2006.00950.x

Richardson, D.M., van Wilgen, B.W. & Nuñez, M.A. 2008. Alien conifer invasions in South America: short fuse burning? *Biological Invasions,* 10, 573-577. DOI: 10.1007/s10530-007-9140-y

Shipley, B. 2010. *From plant traits to vegetation structure: chance and selection in the assembly of ecological communities.* Cambridge, Cambridge University Press.

Simberloff, D. 2013. Introduced Species, Impacts and Distribution of. In: Levin, S.A. (ed.) *Encyclopedia of Biodiversity.* Second edition. Academic Press. 357-368. DOI: https://doi.org/10.1016/B978-0-12-384719-5.00251-3

Stern, J.L., Hagerman, A.E., Steinberg, P.D., Winter, F.C. & Estes, J.A. 1996. A new assay for quantifying brown algal phlorotannins and comparisons to previous methods. *Journal of Chemical Ecology,* 22, 1273-1293.

Sullivan, J.J., Williams, P.A. & Timmins, S.M. 2007. Secondary forest succession differs through naturalized gorse and native kāuka near Wellington and Nelson. *New Zealand Journal of Ecology,* 31(1), 22-38. https://www.jstor.org/stable/24058124 [2021-01-21]

Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. 2007. Let the concept of trait be functional! OIKOS, 116(5), 882-892. DOI: https://doi.org/10.1111/j.0030-1299.2007.15559.x

Warwick, S.I., Thompson, B.K. & Black, L.D. 1987. Genetic variation in Canadian and European populations of the colonizing weed species *Apera Spica-Venti. New Phytologist,* 106, 301-317. DOI: 10.1111/j.1469-8137.1987.tb00145.x

Wheeler, N.C., Guries, R.P. & O´Malley, D.M. 1983. Biosystematics of the genus *Pinus* subsection contortae. *Biochemical Systematics and Ecology,* 11(4), 333-340. DOI: https://doi.org/10.1016/0305-1978(83)90033-9

Whitney, K.D. & Gabler, C.A. 2008. Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. *Diversity and Distributions,* 14(4), 569-580. DOI: https://doi.org/10.1111/j.1472-4642.2008.00473.x

Williamson, M. 1996. *Biological Invasions.* First edition. London, Chapman & Hall

Acknowledgements

I want to start with thanking my main supervisor Michael Gundale for helping me throughout this whole thesis work, beginning to the end. He patiently listened and supported me and taught me many new skills with laboratory work, data analyses and scientific writing. I would also like to thank Susan Nuske who had done a lot of work with the samples prior to my thesis work, which I then could use for my analyses. Her explanations of the sample gathering and prior trait measurements were vital for my work. She also gave valuable advice during the writing process. Also I would like to thank my assistant supervisors Paul Kardol and Marie-Charlotte Nilsson Hegerthorn, who helped me improve my thesis and scientific writing skills during the writing process. Additionally, I want to thank Hilda Edlund for patiently helping me with the statistics and learning R. I would also like to thank all people at the Department of Forest Ecology and Management at SLU that in several ways have helped and supported me in my laboratory work. Lastly, thank you to my family and friends for always being there to listen and support me, it means a lot.

Lisa Lindberg

SENASTE UTGIVNA NUMMER

2019:7 Författare: Jan Lindblad
The future of retention forestry – the historical legacy in stands and its impact on retention in the next generation

2019:8 Författare: Hilda Mikaelsson
Alternative oxidase respiration in the mycorrhizal fungus *Laccaria bicolor*

2019:9 Författare: Joel Jensen
Above- and belowground carbon stocks and effects of enrichment planting in a tropical secondary lowland dipterocarp rainforest

2019:10 Författare: Josefin Runesson
Total carbon sequestration during an entire rotation period of oil palm in northern Borneo

2020:01 Författare: Mikaela Rosendahl
Fysiska och psykiska hälsoeffekter av att vistas i naturen – En pilotstudie utförd på Stora Fjäderägg, Västerbottens län

2020:02 Författare: Jessica Åström
Evaluating abundance of deciduous trees in production forests along small streams – can Sweden meet current policy goals without intensive management

2020:03 Författare: Brita Asplund
5§3 – en statlig storstädning av skogslandskapet

2020:04 Författare: Mikaela Casselgård
Effects of 100 years of drainage on peat properties in a drained peatland forest in northern Sweden

2020:05 Författare: Therese Prestberg
1900- talets skogsbruk i kronoparksskogar – En skogshistorisk studie om Håckren och Bjurfors kronoparker

2020:06 Författare: Nils Södermark
Inverkan av trädslagsval och plantstorlek på tall- och granbestånds anläggningskostnad, skadeutveckling och tillväxt i norra Sveriges kust- och inland

2021:01 Författare: Torben Svensson
Tallsåddens potential för återbeskogning av marker med tjocka humustäcken eller torv i norra Sverige.

2021:02 Författare: Therese Strömvall Nyberg
Vad betyder det att skydda natur? – En europeisk jämförelse av skyddade områden

2021:03 Författare: Oscar Nilzén
The Guardian Forest – sacred trees and ceremonial forestry in Japan

2021:04 Författare: Gustaf Nilsson
Riparian buffer zones widths, windthrows and recruitment of dead wood
A study of headwaters in northern Sweden

2021:05 Författare: Louise Almén
Naturhälsokartan - Hälsofrämjande naturområden i Väster- och Österbotten